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Food Science and Human Wellness 4 (2015) 47–55
Food Science and Human Wellness
www.elsevier.com/locate/fshw

A review: Health promoting lactic acid bacteria in traditional Indonesian fermented foods

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Received 23 November 2014; received in revised form 8 December 2014; accepted 29 January 2015

Abstract

Traditional Indonesian fermented foods can be used as potential sources of probiotics as they commonly contain lactic acid bacteria (LAB), including species of *Lactobacillus*, *Pediococcus*, *Enterococcus*, *Weisella* and *Leuconostoc*. The occurrence of LAB in Indonesian fermented foods is not only limited to lactic fermented foods but is also present in foods with molds as the main starter culture. This review aims to describe the significance of Indonesian fermented foods as potential sources of probiotics and the potential of LAB from fermented foods to promote beneficial health effects. A number of *in vitro* studies have been carried out to assess the probiotic potential of LAB from fermented foods. Many LAB strains have met the basic requirements for them to be considered as probiotics and possess some functional properties contributing to positive health impacts. Hypocholesterolemic effects, stimulation of the immune system, and prevention of diarrhea by some probiotic strains have been shown in animal studies. However, human studies on the efficacy of probiotic strains are still limited. Two strains isolated from dadih, a fermented buffalo milk, are examples of promising probiotic strains that have gone through human studies. The potential probiotic properties of LAB in Indonesian fermented foods still need to be fully investigated to assess their impact on human health. The studies should also consider factors that may influence the functional properties of probiotics, both in foods and in humans.

Keywords: Fermented foods; Probiotics; Diarrhea; Hypocholesterolemic; Antimutagenicity

1. Introduction

Fermentation is known as one of the oldest forms of food preservation in the world. Fermentation can increase the shelf-life of meat, fish, fruit and vegetables that are highly perishable due to their high water contents and nutritive values, especially in tropical countries like Indonesia. Preservation of foods occurs through lactic acid, alcoholic, acetic acid and high salt fermentations. Beside preserving foods, fermentation also changes the organoleptic characteristics of foods through developing a wide diversity of flavors, aromas and textures. Moreover, fermentation may improve digestibility and nutritional quality through enrichment of food substrates with vitamins, proteins, essential amino acids and essential fatty acids [1,2].

As in other parts of East Asia, Indonesian fermented foods feature the use of a variety of raw materials, including cereals, soybeans, fruits, vegetables, tubers and fish. In some parts of Indonesia, meat and milk, especially buffalo milk and mare milk, have been used traditionally as raw materials for fermented products. In terms of the fermentation processes, Indonesian fermented foods can be classified into lactic fermentations (fruits, vegetables, cassava, meat, milk), alcoholic fermentations (rice, cassava), mold fermentations (soybeans, peanut press cake) and high salt fermentations (fish, soy sauce, tauco [fermented soybean slurry]). In the fermentation of some products, such as soy

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Peer review under responsibility of Beijing Academy of Food Sciences.

http://dx.doi.org/10.1016/j.fshw.2015.06.001
2213-4530/

sauce, a mold fermentation is followed by a brine fermentation in which LAB and yeasts are involved [3].

Although some fermentations, such as those for tempe (mold fermented soybean) and tape (alcoholic fermented steamed glutinuous rice or cassava), use a starter culture, microorganisms from the environment may contaminate the ferments and grow during the fermentations. Involvement of microorganisms other than molds in tempe fermentation has started since soaking step and continues during mold fermentation [4,5]. The presence of other microorganisms such as LAB in tape fermentation contributes to the development of flavor of tape [6]. Many Indonesian fermented foods (fruits, vegetables, meat and fish) are produced through natural fermentation by controlling the environment with the addition of salt, or by soaking the raw materials in water, as in the fermentation of raw peeled cassava root. The main role of salt in fruit and vegetable fermentations is to promote the growth of LAB over spoilage bacteria [7,8] and to inhibit pectinolytic and proteolytic enzymes that can cause softening and putrefaction [7].

LAB is a group of Gram-positive, non-spore forming, coccus or rod shaped bacteria. They ferment carbohydrates to almost entirely lactic acid (homofermentation) or to a mixture of lactic acid, carbon dioxide and acetic acid and/or ethanol (heterofermentation). Other compounds, such as diacetyl, acetaldehyde and hydrogen peroxide, are also produced. These compounds contribute to the flavor and texture of fermented foods and may also contribute to the inhibition of undesirable microbes.

The LAB in Asian traditional fermented foods include *Lactobacillus plantarum*, *Lb. pentosus*, *Lb. brevis*, *Lb. fermentum*, *Lb. casei*, *Leuconostoc mesenteroides*, *Leu. kimchi*, *Leu. fallax*, *Weissella confusa*, *W. koreenis*, *W. cibaria*, and *Pediococcus pentosaceus*, many of which are considered to be potential probiotics [7]. Most of the LAB present in Indonesian fermented foods are *Lactobacillus* species (Table 1). Other genera, such as *Pediococcus*, *Lactococcus*, *Enterococcus*, *Weisella* and *Leuconostoc*, are also found in some fermented foods (Table 1). LAB are involved to varying degrees in Asian fermented foods, and may have positive and negative effects on products [9]. In cereal alcoholic fermentations, lactic acid bacteria contribute to the characteristic of flavor and taste. Excessive lactic acid generally lowers the quality of alcoholic fermentation products. However, in fruit, vegetable, milk and meat fermentations, LAB play a major role in producing acid necessary to the quality of the products. It is interesting that LAB are generally present in tempe, which is not an acidic fermentation. In tempe fermentation, soybeans are soaked overnight prior to inoculation with starter culture containing *Rhizopus oligosporus* as the primary microorganism. Acid fermentation involving LAB takes place during the soaking [10,11] and some growth of lactic acid bacteria commonly occurs during the stage of mold growth [5,12].

LAB in fermented foods are of interest not only for their role in fermentation but also for their role in promoting positive health impacts. The concept of beneficial health effects of LAB has existed since Metchnicoff in 1908 proposed that acid producing microorganisms in fermented dairy products could lead to a prolongation of the life span of consumers [36]. Although historically the fermented products associated with beneficial LAB were milk-based, much research has been directed to exploring LAB from other fermented foods as potential probiotics. A probiotic is defined as a live microorganism that will confer beneficial effects on the host when ingested in sufficient amount [37]. The probiotic bacteria used in commercial products are mainly members of the genera *Lactobacillus* and *Bifidobacterium* [36]. Probiotic bacteria are usually those bacteria that have adapted to the gastrointestinal environment. However, recent research has shown promising probiotic activity of LAB isolated from fermented foods [9]. The progress in research on the beneficial health effects of microorganisms, especially LAB, isolated from Indonesian fermented foods is discussed below.

2. Potential of lactic acid bacteria isolated from Indonesian fermented foods as probiotics

Probiotic and other functional properties are strain dependent and all probiotic strains are unique and different; therefore, their properties and characteristics need to be well defined [38]. Several criteria have to be met in selecting probiotic strains, including acid and bile tolerance, survival through the gastrointestinal track, ability to adhere to intestinal surfaces, antimicrobial activity against potentially pathogenic bacteria, and good technological properties [39]. Functional properties of probiotics include hypocholesterolemic activity by lowering plasma cholesterol [40], preventing and treating diarrhea [37], and altering the immune system [41,42]. The mechanisms by which probiotics exert their beneficial effects on the host include the reduction of luminal pH, competition with pathogens for adhesion sites and nutritional sources, secretion of antimicrobial substances, toxin inactivation, and immune stimulation [43].

Based on *in vitro* studies, LAB isolated from Indonesian fermented foods have promising characteristics as probiotic candidates (Table 2). *In vitro* assessment shows that many LAB isolates tolerate bile salt and low pH environment and possess antagonistic activity against foodborne pathogens. These characteristics are similar to those of intestinal microorganisms, such as *Lactobacillus acidophillus* and *Lb. casei* that are commonly used as probiotics [9]. The research results suggest that the LAB in Indonesian fermented food have adapted to environments that resemble the gastrointestinal track and, hence, have potential as probiotic microorganisms. Adaptation of LAB isolated from fermented foods on specific environment such as high salt concentration, acidic condition, has also been reported such as *L. mesenteroides* in Kimchii, cane juice, *Leu. oenos* in grape juice and *Tetragenococcus halophillus* in soy sauce [9]. It has been suggested that adaptation involves the human food cycle, from soil to raw materials, to fermented product, to human intestine, to feces and then to soil again [9].

Table 1
Occurrence of lactic acid bacteria in some Indonesian fermented foods.

Fermented food	Main raw material(s)	Fermentation process	Lactic acid bacteria present	References
Sayur asin	Mustard cabbage leaf	Lactic fermentation	*Lactobacillus farciminis*, *Lb. fermentum*, *Lb. namurensis*, *Lb. plantarum*, *Lb. helveticus*, *Lb. brevis*, *Lb. versmoldensis*, *Lb. casei*, *Lb. rhamnosus*, *Lb. fabifermentans*, *Lb. satsumensis*	[13]
			Leuconostoc mesenteroides, *Lb. confusus*, *Lb. curvatus*, *Pediococcus pentosaceus*, *Lb. plantarum*	[7]
Tempoyak	Flesh of durian (*Durio zibethinus*)	Lactic fermentation	*Lb. plantarum*, *Lb. coryneformis*, *Lb. casei*	[14]
			Lb. plantarum, *Lactobacillus* sp., *Weissella paramesenteroides*, *Pediococcus acidilactici*	[15]
			Enterococcus gallinarum, *E. faecalis*	[16]
Mandai	Flesh of cempedak, a family of jack fruit (*Arthocarphus champeden* Spreg.)	Lactic fermentation	*Lb. plantarum*	[17,18]
			P. pentosaceus	[18]
Tape starter culture	Rice flour	–	*P. pentosaceus*, *E. faecium*, *Lb. curvatus*, *W. confusa*, *W. paramesenteroides*	[19]
Rice wine/rice tape	Glutinuous rice (steamed)	Alcoholic fermentation	*P. pentosaceus*, *Weisella* sp.	[20]
Growol	Cassava (raw)	Lactic fermentation	*Lb. plantarum*, *Lb. rhamnosus*	[21,22]
Tempe	Soybean	Mold fermentation	*Lb. fermentum*, *Lb. plantarum*, *P. pentosaceus*, *W. confusa*, *Lb. delbrueckii* ssp. *delbrueckii*	[23]
			Lb. plantarum	[24]
Soy sauce (kecap)	Soybean	Mold fermentation followed by high salt fermentation (brine fermentation)	*Tetragenococcus halophillus*	[3]
Bakasang	Fish	High salt fermentation	*P. acidilactici*	[25]
Urutan (traditional Balinese sausage)	Lean Pork	Lactic fermentation	*Lb. plantarum*, *P. acidilactici*, *Lb. farciminis*	[26]
Dadih	Buffalo milk	Lactic fermentation	*Leu. mesenteroides*	[27]
			Lactococcus lactis subsp. *lactis*, *Lb. brevis*, *Leu. mesenteroides*, *Lb. casei*	[28]
			Lb. plantarum, *E. faecium*	[29,30]
			Lb. fermentum, *Leu. lactis* subsp. *lactis*	[31]
			Lc. lactis, *Lb. rhamnosus*	[32]
Fermented mare milk	Mare milk	Lactic fermentation	*Lb. rhamnosus*, *Lb. fermentum*	[33,34]
			Lb. acidophilus, *Lb. brevis*	[35]

Adhesion properties similar to those of probiotic strains have also been shown by some isolates from fermented foods, such as *Lactobacillus reuteri* IS-27560, *Lactococus lactis* IS-16183 and *Lb. rhamnosus* IS-7257 from dadih that adhere to mucus layers and Caco-2 cells [32]. *Lc. lactis* IS-16183 and *Lb. rhamnosus* IS-7257 significantly inhibited adhesion of *Escherichia coli* O157:H7. Accordingly, these two strains may be potential candidates for use as probiotic strains. The adhesion properties of dadih isolates were relatively comparable to the commercial probiotic strains, *Lb. casei* Shirota and *Lb. rhamnosus* GG. Another study on dadih isolates (*Lb. plantarum* IS-10506 and IS-20506; *Enterococcus faecium* IS-27526, IS-23427 and IS-16183) showed that *Lb. plantarum* IS-10506 was the most adhesive and can significantly reduce pathogen adhesion to mucus [29]. *Lb. plantarum*, isolated from fermented fruit (mandai), also showed good adhesive properties on enterocyte-like HCT-116 cells, with *Lb. plantarum* MB427 showing the strongest inhibition of adhesion of *Listeria monocytogenes* ATCC 13932, enteropathogenic *E. coli* (EPEC) K1.1 and *Salmonella enterica* serovar Typhimurium ATCC 14028 [17]. These findings suggest that antimicrobial effects of fermented food isolates also involve adhesion properties. Production of substances other than organic acids as shown by certain isolates from bakasang, dadih and growol [25,28,44] may enhance their activity in inhibiting the growth of pathogens.

Among potential health beneficial effects, *in vitro* assessment of fermented foods isolates for their hypocholesterolemic

Table 2
Characteristic and functional properties of lactic acid bacteria isolated from Indonesian fermented foods based on *in vitro* studies.

Lactic acid bacteria	Source of LAB	Characteristic and functional properties	References
Enterococcus gallinarum UP-9, *Enterococcus faecalis* UP-11	Tempoyak	Bile and acid tolerant, being able to conjugate sodium taurocholate	[16]
Lb. plantarum	Mandai	Bile salt and low pH tolerant, good adhesion properties	[17]
Lactobacillus plantarum sa28k	Indonesian sauerkraut	Acid and bile resistant, assimilation of cholesterol	[45]
Lb. acidophilus FNCC116	Moromi soy sauce fermentation		
Lb. casei FNCC262	Tape ketan		
Lb. fermentum	Tempe	Bile salt and low pH tolerant	[23]
Lactobacillus TGR-2	Growol	Inhibits growth of *Staphylococcus aureus*, *S. typhimurium*, *E. coli*, *Bacillus cereus*, *Morganella morganii*. Produces bacteriocin-like compounds	[44]
Lb. casei FNCC262	Tape ketan (alcoholic fermented glutinuous rice)	Tolerance to 0.3% bile salt, resistant to pH 2.5, antimicrobial activity against *E. coli*, *S. aureus*, *B. cereus*	[51]
Lb. acidophillus FNCC 116	Moromi soy sauce (brine fermentation)		
DA-1	Dadih		
DA-2	Dadih		
Lactococcus lactis subsp. *lactis* IS-10285, IS-7386, IS-16183, IS-11857and IS-29862, *Lb. brevis* IS-27560, IS-26958 and IS-23427, *Leuconostoc mesenteroides* IS-27526, and *Lb. casei* IS-7257	Dadih	Bile salt (oxgall), low pH and lysozyme tolerant *Lc. lactis* subsp. *lactis* IS-11857 and IS-29862, *Lb. brevis* IS-26958 showed high alt hydrolase (BSH) activity. *Lc. lactis* subsp. *lactis* IS-10285 and IS-16183 had a positive spectrum of bacteriocin activity against *E. coli* and *Lysteria monocytogenes*	[28]
Lb. plantarum Dad-3	Dadih	Inhibiting *Shigella dysentriae*, *E. coli* and *S. typhi*	[52]
Lb. plantarum Mut 7 and Mut 13	Gatot (fermented cassava)	Inhibiting *Shigella dysentriae*	
Lb. plantarum T-3	Growol (fermented cassava)	Inhibiting *Shigella dysentriae*	
Lb. fermentum I-11 and *Leu. lactis* subsp. *lactis* I-2775	Dadih	Acid and oxgall (bile) tolerant, deconjugated sodium taurocholate and bound cholesterol	[31]
Lb. reuteri IS-27560, *Lc. lactis* IS-16183, *Lb. rhamnosus* IS-7257, *Enterococcus faecium* IS-27526	Dadih	Adherence to mucus layer and Caco-2 cells. IS 16183 and IS7257 inhibit the adhesion of *Escherichia coli* O157:H7 to human intestinal mucosal surface	[32]
Lb. plantarum IS-10506, IS-20506 and *E. faecium* IS-27526, IS-23427 and IS-16183	Dadih	Adhesion was strain dependent with the most adhesive was *Lb. plantarum* strain was IS-10506 and reduced pathogen adhesion to mucus	[29]
Lb. plantarum IS-10507 and *IS-20506*	Dadih	Removal of microcystin-LR, a cyclic heptapeptide hepatotoxin (cyanobacterial toxin)	[47]
Lb.brevis, *Lb.acidophilus*	Fermented mare milk	Survive in low pH (2.5) for 2 h, bile tolerant	[35]
Lb. rhamnosus FSMM15, FSMM22, FSMM26	Fermented mare milk	Bile salt, low pH and artificial gastrointestinal fluids tolerant; and having good adhesion properties	[33]
P. acidilactici	Bekasam/bekasang	Antimicrobial activity against *E. coli*, *S. aureus*, *P. fluorescens*	[24]
LAB (unidentified)	Bekasam	Antimicrobial activity against *E. coli*, *S. typhimurium*, *B. cereus*, *S. aureus*	[53]
Lb. plantarum SMN 025, *Lb. casei* subsp. *rhamnosus* FNCC 098	Fermented foods	β-Glucosidase activity	[48]
Pediococcus acidilactici, *P. pentosaceus*, *P. lolii* AB362985, *Lb. pentosus-plantarum* group	Fermented foods (tape, tempe and fermented vegetables)	β-Glucosidase activity	[49]

effect has been done by some researchers [16,31,45]. Cholesterol binding by LAB in the small intestine may reduce the amount of dietary cholesterol absorbed [31]. Several mechanisms for lowering cholesterol absorption have been hypothesized [46], including enzymatic deconjugation of bile acids by bile-salt hydrolase (BSH), assimilation of cholesterol, co-precipitation of cholesterol with deconjugated bile, binding of cholesterol to the cell walls of probiotic bacteria, incorporation of cholesterol into the cell membranes of probiotics, conversion of cholesterol to coprostanol, and production of short-chain fatty acids by probiotics in the presence of prebiotic substrates. BSH hydrolyzes conjugated glycodeoxycholic acid and taurodeoxycholic acid, leading to the deconjugation of glyco- and tauro-bile acids. Once deconjugated, bile acids are less soluble and absorbed by the intestines, leading to their elimination in the feces. Cholesterol is used to synthesize new bile acids in a homeostatic response, resulting in lowering of serum cholesterol. The ability of cholesterol-binding appeared to be growth and strain specific.

Other potential health benefits shown by LAB isolates from fermented foods are the ability of a *L. plantarum* isolate from dadih to remove microcystin-LR, a cyclic heptapeptide hepatoxin produced by cyanobacteria [47] and the ability to produce β-glucosidase [48,49]. Cyanobacteria produce a number of potent hepato- and neurotoxins, collectively called cyanotoxins, which have potent acute hepatotoxicity and tumor promoting activity [47]. *Lb. plantarum* IS-10506 and IS-20506 have shown ability to efficiently remove the toxins. Meanwhile, β-glucosidase activity is widespread among LAB and presumably plays a role in interactions with the human host [50]. β-Glucosidase releases a wide range of plant secondary metabolites from their β-*D*-glucosylated precursors. The conversion of glucoside isoflavones into their bioactive aglycones by LAB has been observed in soymilk fermentation [48].

LAB isolated from fermented foods has potential as producers of bioactive compounds. Gamma-aminobutyric acid (GABA) has various physiological functions and could be produced by LAB [54]. Some *Lactobacillus* species have been reported to produce equol [7-hydroxy-3-(40-hydroxyphenyl) chroman], a nonsteroidal estrogen of the isoflavone class in fermented soymilk [55].

Most established probiotics are LAB and Bifidobacteria although recently certain yeasts (*e.g. Saccharomyces boulardii* [56–58]) and spore-forming bacteria (*e.g. Bacillus coagulans* [59–61]) have been considered as probiotics. While *S. boulardii* has been shown to be effective in preventing the recurrence of *Clostridium difficile*-induced pseudomembranous colitis as well as the antagonistic action of *E. coli*, the long-term advantages of using spores as probiotics is that they are heat-stable and can survive transit across the stomach barrier, properties that cannot be assured with other probiotic bacteria that are given in the vegetative form [61]. Yeasts are commonly present in many fermented foods [6,11,12,62] and starter culture [63]. Spore-forming bacteria could also be part of the microbial consortium in traditional Indonesian fermented foods, such as tempe [5] and tape [6,64] as well as their starter cultures [62].

3. Health beneficial effect of lactic acid bacteria isolated from fermented foods

3.1. Animal studies

Only a few promising LAB isolated from Indonesian fermented foods have been assessed in animal studies for their potential health benefits (Table 3). Evaluation of *L. plantarum* sa28k, *Lb. acidophilus* FNCC116 and *Lb. casei* FNCC262 in lowering cholesterol in rats, revealed that rats that received milk fermented by the three LAB had significantly lower serum cholesterol levels than rats feeds non-fermented milk [45]. *Lb. acidophilus* KBc and *Lb. brevis* KBa, from fermented mare milk, were able to adhere and colonize gut mucosal epithelium of rats [35]. Administration of these isolates significantly reduced cholesterol levels in blood serum of hypercholesterolemic rabbits. The cholesterol-lowering activity of milk fermented by *Lactococcus lactis* subsp. *lactis* IS-10285 and *Lc. lactis* subsp. *lactis* IS-29862 has been evaluated in hypercholesterolemic rats [65]. The isolates had high taurocholate-deconjugating activity. Only milk fermented by *Lc. lactis* subsp. *lactis* IS-10285 significantly reduced the total serum cholesterol, LDL cholesterol and total bile acids. Neither milk nor fermented milk influenced HDL cholesterol levels. The authors suggested that the hypocholesterolemic effect of *Lc. lactis* subsp. *lactis* IS-10285 was due to its ability to suppress the reabsorption of bile acids into the enterohepatic circulation and enhance the excretion of bile acids in feces of hypercholesterolemic rats. These results indicate that strains of LAB isolated from Indonesian fermented foods could be considered as probiotic strains that have beneficial effects in reducing serum cholesterol levels.

Candidate probiotic strains isolated from Indonesian fermented foods have also been evaluated in the treatment or prevention of diarrhea caused by Enteropathogenic *E. coli* (EPEC) infection. *Lb. plantarum* MB427 from mandai at 10^9 cfu/mL reduced the incidence and severity of diarrhea and shortened the duration of diarrhea in EPEC-induced diarrhea in Sprague-Dawley rats [17] and increased secretion of serum IgA and IgB. Supplementation of dadih isolate, *P. pentosaceus*, at a dose of 2×10^8 cfu/g reduced stool frequency, lowered tumor necrosis factor-α levels and improved the balance of gut microflora in EPEC-induced diarrheal mice (*Mus muscullus*) [66]. Similar results were observed with a *W. paramesenteroides* strain also isolated from dadih [67]. The researchers suggested that probiotic supplementation may protect against mucosal epithelial cell damage by *E. coli* exposure and protect cell against further damage by TNF-α and interferon (IFN)-γ. Probiotics are able to downregulate T helper (Th)-1 responses and inhibit the production of proinflammatory cytokines, such as TNF-α by dendritic cells. The decreased the concentration of TNF-α in the feces, decreased the serum levels of TNF-α, and stool frequency. These studies indicate that potential probiotic bacteria isolated from fermented foods could improve the immune system and reduce diarrhea incidents. Diarrhea is the third leading cause of death, following tuberculosis and pneumonia, in Indonesia [66].

Table 3
Health benefits of lactic acid bacteria isolated from Indonesian fermented foods as assessed in animal studies.

Lactic acid bacteria	Source of LAB	Characteristic and functional properties	References
Lb. plantarum MB 427	Mandai	Shortened duration of diarhea in rats caused by EPEC infection and induced secretion of IgA and IgG	[17]
Lactobacillus plantarum sa28k *Lb. acidophilus* FNCC116 *Lb. casei* FNCC262	Indonesian sauerkraut Moromi soy sauce fermentation Tape ketan	Assimilating cholesterol and lowering serum cholesterol in rats	[45]
Lb. acidophilus KBc and *Lb. brevis* Kba	Fermented mare milk	Adhered to and colonized gut mucosa epithelium of rat, reduced cholesterol level of blood serum of rabbits with hypercholesterolemia condition	[35]
Lactococcus lactis subsp. *lactis* IS-10285	Dadih	Significantly reduced serum total cholesterol, LDL cholesterol and total bile acids in hypercholesterolemic rats	[65]
P. pentosaceus	Dadih	Reduced stool frequency, lowered TNF-α level and improved the balance of gut microflora in EPEC-induced diarrheal mice	[66]
W. mesenteroides	Dadih	Reduce stool frequency, lower TNF-α level and improve the balance of gut microflora in EPEC-induced diarrhea mice	[67]
Enterococcus faecium IS-27526	Dadih	Significantly lowered fecal mutagenicity of rats fed with milk cultured with the isolate	[30]

The most common causes of diarrhea in children, both in developed and developing countries are *E. coli*, Rotavirus, *Salmonella* spp., *Shigella* spp., *Campylobacter jejuni*, *Entamoeba histolytica* and *Giardia lamblia* [68]. The promising features of LAB isolated from fermented foods in reducing/preventing the incident of diarrhea needs to be confirmed in human studies.

Modification of gut bacterial activities has also correlated with antimutagenicity. Potential probiotic bacterium, *E. faecium* IS-27526 isolated from dadih, showed *in vivo* anti-mutagenic properties toward Trp-P1 of rats [30]. Milk cultured with *E. faecium* IS-27526 significantly lowered fecal mutagenicity and the recovery of Trp-P1 in urine was significantly lower than in control rats fed skim milk. The anti-mutagenic properties was considered due to binding ability of the bacterial cell wall of dadih lactic cultures toward chemicals mutagen.

3.2. Human studies

Human studies on LAB isolated from Indonesian fermented foods are very limited. Two potential probiotics originating from dadih have gone through a clinical study in humans [69,70]. Surono et al. [69] evaluated the effect of *E. faecium* IS-27526 in milk on humoral immune response and on body-weight of young children aged between 15 and 54 months. A 90 days randomized, double-blind, placebo-controlled study was conducted with two groups of young children, placebo and probiotic group. Ultra high temperature treated, low fat milk was used as the carrier of 2.3×10^8 cfu/day of the probiotic. The results showed that *E. faecium* IS-27526 had a significant positive effect on humoral immune response and salivary IgA in underweight young children, and on their weight gain. However, the total serum IgA did not significantly increase in the probiotic group compared with the placebo group.

Another human study [70] evaluated the effect of probiotic *L. plantarum* IS-10506 (originally isolated from dadih) and zinc supplementation on humoral immune response and zinc status of Indonesian infants aged 12–24 months in a 90-day randomized, double-blind, placebo-controlled trial. A combination of microencapsulated *Lb. plantarum* IS-10506 at a dose of 10^{10} cfu/day and 8 mg of elemental zinc showed a potential ability to improve the zinc status of the infants. Supplementation with the probiotic and zinc resulted in a significantly increased humoral immune response, as well as improved zinc status. The effects of probiotics on the humoral immune response was suggested to be the result of colonization and adhesion to epithelial cells, and production of SIgA production induced by the cell wall component of probiotics, such as lipoteichoic acids and peptidoglycan [69]. Both strong immune responses and gut integrity play important roles in the repair of intestinal brush border damage as a result of the balance of microbiota. Zinc and probiotics work *via* different mechanisms, but they are possible to have a synergistic effect. While zinc is necessary for the activity of some immunity mediators, probiotic supplementation could improve the integrity of the intestine, which in turn will optimize the absorption of zinc. The researchers considered that the health beneficial effects of zinc and probiotics could be amplified when they are taken together, more specifically, resulting in improved mineral absorption or a higher cellular immune response.

4. Future perspectives

Many traditional Indonesian fermented foods are potential sources of microorganisms, especially LAB, with promising beneficial health effects. Among the functional properties explored, LAB originating from Indonesian fermented foods showed promising effects on hypocholesterolemia, stimulation of the immune system, and in the prevention of diarrhea. These beneficial health effects could contribute to promoting the health status of Indonesians. However, the functional properties of many promising LAB originating from Indonesian fermented foods have not been fully investigated. Studies are mainly of a preliminary nature and need to be confirmed in animal and human studies. Although most Indonesian fermented foods are historically safe, exploiting a single microorganism should also be supported with studies to confirm its safety, especially for strains belonging to genera that have been reported to pose safety issues. In addition, the ability of LAB of to produce bioactive compounds is an area that needs to be explored extensively in order to maximize the beneficial health impact.

Consumers are aware that fermented foods contain microorganisms and, hence, they become suitable matrixes as probiotic carriers. Among Indonesian fermented products, tempe, as a cheap meat substitute, is an important part of the daily diet. However, tempe is commonly consumed after cooking, which would kill any live probiotic LAB. Other fermented foods, such as dadih, tape, sayur asin and beverages, that are consumed without cooking are more suitable as probiotic carriers. When fermented foods are used as carriers of probiotic bacteria, factors that may influence the ability of the probiotic to survive in the product, during fermentation and marketing, and their activity when entering the human gastrointestinal track, must be considered. LAB with beneficial health effects in fermented foods could serve both as probiotics and as the fermentative organism. Ideally, probiotic cultures to be incorporated into fermented products should have multifunctional characteristics. They should be able to grow and ferment the product, or at least not have any negative impact on the organoleptic properties of the food, while maintaining their probiotic properties.

Exploration of potentially beneficial microorganisms in Indonesian fermented foods needs to be extended to microorganisms other than LAB that are also present in fermented foods. Yeasts and some species of *Bacillus* for examples are the potential microorganisms for probiotic.

Traditional Indonesian fermented foods, utilizing a variety of raw materials, have been handed down for many generations. Although challenges remain, the microorganisms present and involved during fermentation as well as their metabolic products may contribute health benefits to consumers. Therefore, more evidence on the health impacts of beneficial microorganisms and understanding of the relationships between fermented foods, beneficial microorganisms and human health are essential if use of beneficial microorganisms to promote health is to be fully exploited.

Acknowledgement

The author would like to thank Dr. J.D. Owens of University of Reading, UK for his assistance in reading and correcting the manuscript.

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